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(71) Applicant: THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, U.S. DEPARTMENT OF COMMERCE [US/US]; 5285 Port Royal Road, Springfield, VA 22161 (US). (72) Inventors: GINNS, Edward, I. ; 8617 Rayburn Road, Bethesda, MD 20817 (US). MARTIN, Brian ; 5608 Lone Oak Drive, Bethesda, MD 20184 (US). MAY-SACK, Kara, A. ; 2601 Spencer Road, Chevy Chase, MD 20815 (US). ELIASON, William, K. ; 2258 Gunsmith Square, Reston, VA 22091 (US). LaMARCA, Mary, E. ; 5127 Baltimore Avenue, Bethesda, MD 20816 (US).		Published <i>With international search report.</i>
(54) Title: CLONED DNA FOR SYNTHESIZING UNIQUE GLUCOCEREBROSIDASE		
(57) Abstract A cloned cDNA for synthesis of unique glucocerebrosidase is provided. The enzyme thus produced is different from heretofore known similar enzymes.		

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-1-

1 CLONED DNA FOR SYNTHESIZING UNIQUE
2 GLUCOCEREBROSIDASE

3 BACKGROUND OF THE INVENTION

4 Technical Field:

5 The present invention is related to the
6 construction of an expression vector for the synthesis of
7 a recombinant enzyme. More particularly, the present
8 invention is related to the large scale production of
9 glucocerebrosidase by infecting invertebrate cells with a
10 recombinant baculovirus containing the complete cDNA
11 sequence for encoding glucocerebrosidase.

12 State of the Art:

13 Mutation or deficiency of the lysosomal
14 glycoprotein glucocerebrosidase (EC 3.2.1.45,
15 β -D-glucosyl-N-acylsphingosine glycohydrolase) results in
16 Gaucher's disease. It is estimated that there are about
17 20,000 cases of this genetic disease in the U.S. alone.

18 Published methods for producing large quantities
19 of the active human enzyme involve purification of the
20 protein from large amounts of human tissue, such as
21 placenta. It should be noted, however, that the
22 placental glucocerebrosidase has carbohydrate structure
23 different than that of the enzyme found in human liver,
24 spleen, brain or macrophages.

- 2 -

1 Construction of a cDNA clone containing the entire
2 human glucocerebrosidase coding region has been known
3 (Sorge, et al., Proc. Natl. Acad. Sci. USA, 82:7289-7293,
4 1985). However, as it will become clear vide infra, both
5 the cDNA clone of the present invention and the enzyme
6 synthesized therefrom, are qualitatively different from
7 the similar prior art entities.

8 SUMMARY OF THE INVENTION

9 It is, therefore, an object of the present
10 invention to provide an active clone of human cDNA
11 containing the complete coding region for the lysosomal
12 glycoprotein glucocerebrosidase (GCS), preferably
13 introduced into the genome of Autographa californica
14 nuclear polyhedrosis virus downstream to the polyhedrin
15 promoter.

16 It is a further object of the present invention to
17 provide synthetic, isolated and substantially pure
18 recombinant GCS in which the carbohydrate moiety in the
19 glycoprotein structure is different from the human
20 placental GCS.

21 It is another object of the present invention to
22 provide a method for large scale production of
23 recombinant GCS by infecting Spodoptera frugiperda cells
24 with the recombinant vector of the present invention.

25 It is a still further object of the present
26 invention to provide a method for treating Gaucher's
27 disease comprising administering to a subject inflicted
28 with Gaucher's disease, therapeutic amount of the
29 recombinant GCS to alleviate the disease condition.

30 Other objects and advantages of the present
31 invention will become apparent from the following
32 Detailed Description of the Invention.

- 3 -

BRIEF DESCRIPTION OF THE DRAWINGS

These and other objects, features and many of the attendant advantages of the invention will be better understood upon a reading of the following detailed description when considered in connection with the accompanying drawings wherein:

Fig. 1 shows DNA sequence of a human glucocerebrosidase cDNA used for the construction of the baculovirus derived vector, pAC373/GC. In addition to the nucleotide sequence, the amino acids encoded by the coding sequence of the cDNA for human lysosomal glucocerebrosidase is also shown;

Fig. 2 shows schematic construction of baculovirus derived vector containing cDNA for human glucocerebrosidase. The cDNA for human glucocerebrosidase containing the sequence shown in Figure 1 was blunted and then ligated into the SmaI site of a pUC vector (for instance pUC19) yielding pUC19/GC with the cDNA for human glucocerebrosidase lying between unique EcoRI and XbaI sites. The human glucocerebrosidase cDNA could be excised with EcoRI and XbaI, blunted, and ligated in a blunted BamHI site in the baculovirus derived vector, pAC373/GC. This baculovirus vector construct, pAC373/GC, contains human glucocerebrosidase cDNA downstream from the polyhedrin promoter in a 5' to 3' orientation;

Fig. 3 shows comparative data of pH profiles of human placental glucocerebrosidase and the recombinant enzyme produced by using the baculovirus expression system. The recombinantly produced human glucocerebrosidase in both the cell pellet and the culture supernatant has a broad range of pH activity

- 4 -

1 (between pH 3.5 and pH 8.0) with pH optima at
2 approximately pH 4.5 and pH 5.5. The human placental
3 enzyme has a broad range of pH activity (between pH 3.5
4 and pH 8.0) with pH optima at approximately pH 5.0 and pH
5 6.0;

6 Fig. 4 presents comparative results by Western
7 blot analysis of human placental glucocerebrosidase and
8 the recombinant human glucocerebrosidase produced in the
9 eucaryotic SF9 cells. Sample preparation,
10 electrophoresis and Western blot analysis were performed
11 as described in the text. Molecular weight size markers
12 ($M_r \times 10^{-3}$) were phosphorylase b, albumin, ovalbumin, and
13 carbonic anhydrase. P, placental enzyme; M, media
14 glucocerebrosidase; and C, cell-associated
15 glucocerebrosidase. Western blot from untreated
16 placental enzyme and recombinantly produced protein are
17 shown in lanes P1 and, M4 and C7, respectively. Cross
18 reactive material (CRM) from endoglycosidase-H and
19 N-glycanase digested samples are shown in lanes P2, M5,
20 C8, and P3, M6, C9, respectively.

21 DETAILED DESCRIPTION OF THE INVENTION

22 The above and various other objects and advantages
23 of the present invention are achieved by a cDNA clone
24 containing the complete coding sequence for human
25 lysosomal glucocerebrosidase as shown in Figure 1, said
26 clone having been inserted into the genome of Autographa
27 californica nuclear polyhedrosis virus downstream to the
28 polyhedrin promoter as shown schematically in Figure 2.

29 Unless defined otherwise, all technical and
30 scientific terms used herein have the same meaning as
31 commonly understood by one of ordinary skill in the art

- 5 -

1 to which this invention belongs. Although any methods
2 and materials similar or equivalent to those described
3 herein can be used in the practice or testing of the
4 present invention, the preferred methods and materials
5 are now described. All publications mentioned hereunder
6 are incorporated herein by reference.

7 The term "substantially pure" as defined herein
8 means as pure as can be obtained by standard purification
9 techniques known to one of ordinary skill in the art.

10 MATERIALS AND METHODS

11 Materials: Restriction endonucleases and
12 recombinant enzymes were obtained from either Life
13 Science Technologies or New England Biolabs.
14 Concanavalin A-Sepharose was obtained from Pharmacia.
15 Octyl-Agarose and Decyl-Agarose were purchased from ICN
16 Biomedicals, Inc. Polyvinylidene difluoride (PVDF)
17 membranes, 0.45 μ m pore size, were obtained from
18 Millipore Corp. Sequencer chemicals and solvents for
19 on-line PTH analysis were purchased from Applied
20 Biosystems Inc. Endoglycosidase H was from Miles
21 Scientific while N-Glycanase was purchased from Genzyme
22 Corp.

23 Construction of recombinant Baculoviruses:
24 Spodoptera frugiperda SF9 cells, plasmid pAc373, and
25 wild-type AcNPV strain E2 were obtained from Max Summers,
26 Texas A&M University. The SF9 cells were maintained in
27 culture at 28°C using TNM-FH media (GIBCO) (Hink, Nature,
28 226:466, 1970). The cDNA for human glucocerebrosidase
29 was obtained from plasmid pUC19/GC, a derivative of an
30 Okayama-Berg clone from a SV40 transformed human
31 fibroblast cDNA library (Okayama, et

- 6 -

1 al., Mol. Cell Biol., 3:280, 1983). This cDNA contained
2 5' and 3' untranslated sequences as well as the complete
3 coding region for glucocerebrosidase. As shown in Figure
4 2, pAc373/GC was generated by ligation of the blunted
5 EcoRI-XbaI fragment from pUC19/GC into the blunted unique
6 BamHI site of pAc373. Correct orientation of the
7 inserted glucocerebrosidase cDNA was determined by
8 restriction endonuclease analysis.

9 Recombinant baculovirus containing the human
10 glucocerebrosidase coding sequence under transcriptional
11 control of the polyhedrin promoter was produced by
12 cotransfection of wild-type virus, AcNPV, with plasmid
13 pAc373/GC into SF9 cells as described by Summers, et al.,
14 (Tex. Agric. Exp. Stn. Bull. No. 1555, 1987). Five to
15 six days after cotransfection, virus was harvested from
16 the culture supernatant and used to inoculate new
17 monolayers of SF9 cells in petri dishes that were
18 subsequently overlaid with 1% low melting agarose
19 containing TNM-FH medium. Seventy-two hours later the
20 agarose overlay was removed and stored at 4°C, and the
21 cell monolayer was blotted onto a nitrocellulose disk
22 (BA85, Schleicher & Schuell). The disk was hybridized to
23 the random primed, ³²P labelled EcoRI-XbaI
24 glucocerebrosidase cDNA fragment from pUC19/GC. Areas on
25 the agarose overlay corresponding to points on the
26 nitrocellulose disk showing hybridization signal were
27 excised and placed in one milliliter of TNM-FH medium.
28 This virus was used for infection of SF9 monolayer
29 cultures and an additional 5 cycles of
30 infection-hybridization were carried out during the
31 plaque purification procedure.

32 A deposit of pAc373/GC has been made at the ATCC,
33 Rockville, Maryland on November 30, 1987 under the

- 7 -

1 accession number 40393. The deposit shall be viably
2 maintained, replacing if it became non-viable, for a
3 period of 30 years from the date of the deposit, or for 5
4 years from the last date of request for a sample of the
5 deposit, whichever is longer, and made available to the
6 public without restriction in accordance with the
7 provisions of the law. The Commissioner of Patents and
8 Trademarks, upon request, shall have access to the
9 deposit.

10 Enzyme purification: Recombinantly produced
11 glucocerebrosidase was isolated using a modification of
12 the procedure described by Furbish, et al., (Proc. Natl.
13 Acad. Sci. USA, 74:3560, 1977). Cell culture
14 supernatants were precipitated with 195 gm/liter ammonium
15 sulfate. SF9 cell pellets containing the recombinantly
16 produced glucocerebrosidase were extracted into 20
17 milliliters of sodium phosphate buffer, pH 6.5,
18 containing 150 mM NaCl and 0.1% Triton X-100, followed by
19 sonication twice at 50W for 10 seconds. After
20 precipitation with ammonium sulfate (195 gm/liter) the
21 resuspended pellets were extracted with n-butanol, but
22 ultrafiltration using a YM30 membrane (Amicon) replaced
23 dialysis. After decyl-agarose and octyl-agarose
24 hydrophobic interaction chromatography at room
25 temperature (about 22°-25°C), the fractions containing
26 glucocerebrosidase activity were pooled, and the ethylene
27 glycol concentration reduced using an Amicon
28 ultrafiltration cell fitted with a YM30 membrane.

29 Substantially pure enzyme is then obtained
30 following standard conventional purification techniques
31 well known in the art.

32 Carbohydrate characterization. Endoglycosidase-H
33 was dissolved in 100 mM sodium acetate, pH 6.0, at a

- 8 -

1 final concentration of 10 units/ml. N-glycanase was
2 supplied as a 250 unit/ml suspension in 50% glycerol.
3 Either human placental enzyme or fifty microliter aliquot
4 of decyl-agarose fraction containing glucocerebrosidase
5 activity were adjusted to 0.5% NaDodSO₄/1M
6 β -mercaptoethanol and boiled for two minutes. The
7 samples were then diluted with appropriate buffer to
8 either 200 mM sodium acetate, pH 6.0 (for
9 endoglycosidase-H) or 200 mM sodium phosphate, pH 8.5
10 (for N-glycanase) to a final composition of 0.1% SDS,
11 0.7% NP-40, and 0.02M β -mercaptoethanol. The samples
12 were again boiled for 1 min and then either
13 endoglycosidase-H or N-glycanase added to final
14 concentrations of 50 mu/ml or 20 U/ml, respectively.
15 Digestions were for about 16 hours at 37°C.
16 Carboxypeptidase Y was used as a control for both
17 deglycosylation reactions.

18 Western blot analysis: NaDodSO₄ polyacrylamide
19 gel electrophoresis and Western blot analysis were
20 performed as described by Ginns, et al., (Proc. Natl.
21 Acad. Sci. USA, 79:5607, 1982).

22 Amino acid sequence analysis: Samples used for
23 amino acid sequence analysis were electrophoretically
24 fractionated on NaDodSO₄ polyacrylamide gels as described
25 above and then transferred to PVDF membranes as described
26 by Matsudaira (J.B.C., 262:10035, 1987). Typically,
27 after electrophoresis the gel was incubated in transfer
28 buffer (0.01M CAPS, 10% methanol, pH 11.0) for 10 minutes
29 prior to transblotting (50 ma for 4 hours). The gel was
30 then washed with HPLC grade water for 5 minutes, stained
31 with 0.1% Coomassie Blue R250 (in 50% methanol) for 5
32 minutes, and finally destained for 10 minutes with 50%
33 methanol-10% acetic acid. The PVDF

- 9 -

1 membrane was again washed with HPLC grade water, dried
2 under a stream of nitrogen and stored in a sealing bag at
3 -20°C until used for amino acid sequencing.

4 Amino acid sequence analysis was accomplished
5 using an Applied Biosystems Model 470A gas-phase
6 sequencer equipped with a Model 120A on-line PTH-amino
7 acid analyzer. The program 03R PTH was used directly
8 for sequencing without pretreatment of the membrane strip
9 with polybrene. An approximately 2 x 8 mm piece of PVDF
10 membrane containing the protein band of interest was
11 excised, centered on the teflon seal, and placed in the
12 cartridge block of the sequencer. Multiple strips of the
13 PVDF membrane could be stacked in this manner, thus
14 increasing the amount of protein available for
15 sequencing. The initial and repetitive yields for
16 sequencing recombinant glucocerebrosidase were calculated
17 by comparison with the yields obtained after 100
18 picomoles of human placenta glucocerebrosidase were
19 electrophoresed, transblotted to PVDF and subjected to
20 ten cycles of amino acid sequence (Table 1).

21 Table 1 compares the N-terminal amino acid
22 sequence of mature human placental glucocerebrosidase to
23 N-terminal amino acid sequence of recombinant human
24 glucocerebrosidase using the methods described in the
25 text. The N-terminal amino acids determined by direct
26 chemical sequencing of the mature human placental and
27 recombinant glucocerebrosidase are identical indicating
28 that the signal sequence in the recombinantly produced
29 enzymes are correctly processed. The blank in amino acid
30 position 4 of the recombinant enzyme sequence is
31 consistent with cysteine because cysteine was only
32 identified in the placental enzyme following reduction
33 and alkylation of the protein. The vertical arrow above

- 10 -

- 1 the human cDNA sequence indicates the site of peptidase
- 2 cleavage of the signal sequence.

TABLE 1

ATG GCT GGC.....TCA GGT [↓] GCC CGC CCC TGC ATC CCT AAA AGC TTC GGC :cDNA
M A G S G A R P C I P K S F G

A R P C I P K S F G :placental enzyme

A R P - I P K S F G :culture media

A R P - I P K S F G :SF9 cell pellet

- 11 -

1 Glucocerebrosidase assays: For pH profile and
2 inhibition studies, glucocerebrosidase activity was
3 measured using 100 mM potassium phosphate buffer
4 containing 0.15% Triton X-100, 2.5 μ l of β -D-[1- 14 C]
5 glucocerebroside (7.5 mg/ml in sodium taurocholate at
6 50 mg/ml), and the sample in the total volume of 200 μ l.
7 Preincubations with conduritol-B-epoxide were for 30 min
8 at 37°C. For K_m determination, β -glucosidase activity
9 was assayed at pH 5.9 using the artificial substrate
10 4-methylumbelliferyl- β -D-glucopyranoside (4MUGP) in 100 mM
11 potassium phosphate buffer containing 0.15% Triton X-100
12 and 0.125% sodium taurocholate. Purification of
13 recombinant glucocerebrosidase was also monitored using
14 4MUGP.

15 Figures 1-4 and Table 1 show the comparative
16 results demonstrating the distinctive nature and
17 properties of the cDNA clone and GCS of the present
18 invention relative to the other known similar clones and
19 enzymes, particularly comparing Sorge et al clone and
20 placenta enzyme.

21 The distinctive properties are listed below:

22 (1) The human cDNA of the present invention for
23 glucocerebrosidase differs in both nucleotide sequence
24 and translated amino acid sequence from that of Sorge, et
25 al., (PNAS, 1985, and Correction PNAS, 1986).
26 Specifically, the cDNA of the present invention encodes
27 for Leu (at 489) and Arg (at 514) while that of Sorge, et
28 al., encodes Pro and His at position 489 and 514,
29 respectively. In addition, this cDNA sequence differs in
30 three nucleotides from that reported by Tsuji, et al.,
31 (J.B.C., 261:50, 1986).

- 12 -

1 (2) The high level baculovirus expression system
2 differs from other expression systems as should be known
3 to those familiar with the subject. For example, the
4 proteins expressed using bacterial hosts do not have the
5 carbohydrate moieties that are added by eukaryotic
6 hosts. Transient expression systems utilizing COS cells
7 or L cells produce only about 200,000 Units
8 glucocerebrosidase/liter (Choudary, et al., 1986) while
9 the Baculovirus expression system produces over 2,400,000
10 units glucocerebrosidase/liter after three days of
11 culture. Similarly, enzyme produced in heterologous
12 cells following retroviral gene transfer produces
13 approximately 200,000 units glucocerebrosidase/liter
14 (Choudary, et al., 1986, Cold Spring Harbor Symposia, Vol
15 LI: 1047).

16 (3) The purification of human glucocerebrosidase from
17 large amounts of human placenta must take into account
18 the risk of the possible presence of infectious agents
19 (such as but not limited to AIDS virus and hepatitis
20 virus). The recombinantly produced glucocerebrosidase is
21 not associated with these potential complications.

22 (4) The carbohydrate structure of glucocerebrosidase
23 isolated from human placenta is different from that of
24 recombinantly produced glucocerebrosidase by the
25 baculovirus system (see Figure 4).

26 (5) Several biochemical parameters of the human
27 placental enzyme are different than that of the
28 recombinant glucocerebrosidase produced by employing the
29 baculovirus expression system:

- 13 -

1 1) The human placental enzyme on Western blot
2 analysis showed a major band of cross reactive
3 material (CRM) at 65 kDa (see Figure 4), while the
4 recombinantly produced enzyme has multiple CRM
5 forms between 67 and 52 kDa. However, upon
6 enzymatic removal of carbohydrate, both the
7 recombinantly produced and placental enzyme has a
8 single major CRM form at 52 kDa.

9 2) The recombinant enzyme was active between pH
10 3.5 and pH 8.0 with pH optima at pH 4.5 and pH
11 5.5. The human placental enzyme was active
12 between pH 3.5 and pH 8.0 with pH optima at pH 5.0
13 and pH 6.0 (see Figure 3).

14 3) The recombinantly produced enzyme in the
15 media and cell pellet have K_m 's of 3.3 mM and 3.6
16 mM. respectively. the K_m for the placental enzyme
17 is reported to be 8 mM (Basu, et al., J.B.C.,
18 259:1714, 1984).

19 It is clear from the above that the recombinantly
20 produced GCS of the present invention is a quantitatively
21 different protein than any other heretofore known entity.

22 Since the carbohydrate pattern of the
23 recombinantly produced GCS of the present invention is
24 more like that of the human liver, spleen, brain or
25 macrophage GCS, as compared to the placental enzyme and
26 obtained in large quantities by the expression vector of
27 the present invention, replacement therapy of Gaucher's
28 disease now becomes possible for treating patients
29 afflicted with this disease. A method of treating this
30 disease comprises administering to a subject afflicted

- 14 -

1 with Gaucher's disease, therapeutic amounts of
2 recombinant GCS of the present invention to alleviate
3 said disease condition.

4 A pharmaceutical composition comprises therapeutic
5 amounts of the GCS of the present invention and
6 pharmaceutically acceptable carrier such as physiological
7 saline, non-toxic sterile buffers and the like.

8 It is understood that the examples and embodiments
9 described herein are for illustrative purposes only and
10 that various modifications or changes in light thereof
11 will be suggested to persons skilled in the art and are
12 to be included within the spirit and purview of this
13 application and scope of the appended claims.

- 15 -

1 WHAT IS CLAIMED IS

- 2 1. A cDNA clone containing complete coding
3 region for human lysosomal glycoprotein
4 glucocerebrosidase, said coding region comprising
5 nucleotide sequence as follows:

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G GAG TTT TCA AGT CCT TCC AGA GAG GAA TGT CCC AAG CCT TTG AGT
AGG GTA AGC ATC ATG GTC GGC AGC CTC ACA GGA TTG CTT TCA CTT CAG
GCA GTG TCG TGG CGA TCA GGT GCC CGC CCC TGC ATC CCT AAA AGC TTC
GGC TAC AGC TCG GTG GTG TGT GTC TGC AAT GCC ACA TAC TGT GAC TCC
TTT GAC CCC CGC ACC TTT CCT GGC CTT GGT ACC TTC AGC CGC TAT GAG
AGT ACA CGC AGT GGG CGA GCG ATG GAG CTG AGT ATG GGG CCC ATC CAG
GCT AAT CAC ACG GGC ACA GGC CTG CTA CTG ACC CTG CAG CCA GAA CAG
AAG TTC CAG AAA GTG AAG GGA TTT GGA GGG GCC ATG ACA GAT GCT GCT
GCT CTC AAC ATC CTT GCC CTG TCA CCC CCT GCC CAA AAT TTG CTA CTT
AAA TCG TAC TTC TCT GAA GAA GGA ATC GGA TAT AAC ATC ATC CGG GTA
CCC ATG GCC AGC TGT GAC TTC TCC ATC CGC ACC TAC ACC TAT GCA GAC
ACC CCT GAT GAT TTC CAG TTG CAC AAC TTC AGC CTC CCA GAG GAA GAT
ACC AAG CTC AAG ATA CCC CTG ATT CAC CGA GCC CTG CAG TTG GCC CAG
CGT CCC GTT TCA CTC CTT GCC AGC CCC TGG ACA TCA CCC ACT TGG CTC
AAG ACC AAT GGA GCG GTG AAT GGG AAG GGG TCA CTC AAG GGA CAG CCC
GGA GAC ATC TAC CAG CAG ACC TGG GCC AGA TAC TTT GTG AAG TTC CTG
GAT GCC TAT GCT GAG CAC AAG TTA CAG TTC TGG GCA GTG ACA GCT GAA
AAT GAG CCT TCT GCT GGG CTG TTG AGT GGA TAC CCC TTC CAG TGC CTG
GCC TTG ACC CCT GAA CAT CAG CGA GAC TTC ATT GCC CST GAC JTA GGT
CCT ACC CTC GCC AAC AGT ACT CAC CAC AAT GTC CGC CTA CTC ATG CTG
GAT GAC CAA CGC TTG CTG CTG CCC ACC TGG GCA AAG GTG GTA CTG ACA
GAC CCA GAA GCA GCT AAA TAT GTT CAT GGC ATT GCT GTA CAT TGG TAC
CTG GAC TTT CTG GCT CCA GCC AAA GCC ACC CTA GGG GAG ACA CAC CGC
CTG TTC CCC AAC ACC ATG CTC TTT GCC TCA GAG GCC TGT GTG GGC TCC
AAG TTC TGG GAG CAG AGT GTG CGG CTA GGC TCC TGG GAT CGA GGG ATG
CAG TAC AGC CAC AGC ATC ATC ACG AAC CTC CTG TAC CAT GTG GTC GGC
TGG ACC GAC TGG AAC CTT GCC GTG AAC CCC GAA GGA GGA CCC AAT TGG
GTG CGT AAC TTT GTC GAC AGT CCC ATG ATT GTA GAC ATC ACC AAG GAC
ACG TTT TAC AAA CAG CCC ATG TTC TAC CAC CTT GGC CAC TTC AGC AAG
TTC ATT CCT GAG GGC TCC CAG AGA GTG GGG CTG GTT GCC AGT CAG AAG
AAC GAC CTG GAC GCA GTG GCA TTG ATG CAT CCC GAT GGC TCT GCT GTT
GTG GTG GTG CTA AAC CGC TCC TCT AAG GAT GTG CCT CTT ACC ATC AAG
GAT CCT GCT GTG GGC TTC CTG GAG ACA ATC TCA CCT GGC TAC TCC ATT
CAC ACC TAC CTG TGG CGT CGC CAG TGA TGG AGC AGA TAC TCA AGG AGG
CAC TGG GCT CAG CCT GGG CAT TAA AGG GAC A
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2. The clone of claim 1 inserted into
baculovirus genome.

- 16 -

1 3. The clone of claim 2 directing synthesis of
2 unique glucocerebrosidase when Spodoptera frugiperda
3 cells are infected therewith.

4 4. Isolated, substantially pure synthetic
5 glycoprotein glucocerebrosidase wherein carbohydrate
6 structure of said glycoprotein is different from human
7 placental glucocerebrosidase.

8 5. The glycoprotein having the following amino
9 acid sequence:

Met Ala Gly Ser Leu Thr Gly Leu Leu Leu Leu Gln
Ala Val Ser Trp Ala Ser Gly Ala Arg Pro Cys Ile Pro Lys Ser Phe
Gly Tyr Ser Ser Val Val Cys Val Cys Asn Ala Thr Tyr Cys Asp Ser
Phe Asp Pro Pro Thr Phe Pro Ala Leu Gly Thr Phe Ser Arg Tyr Glu
Ser Thr Arg Ser Gly Arg Arg Met Glu Leu Ser Met Gly Pro Ile Gln
Ala Asn His Thr Gly Thr Gly Leu Leu Leu Leu Gln Pro Glu Gln
Lys Phe Gln Lys Val Lys Gly Phe Gly Gly Ala Met Thr Asn Ala Ala
Ala Leu Asn Ile Leu Ala Leu Ser Pro Pro Ala Gln Asn Leu Leu Leu
Lys Ser Tyr Phe Ser Glu Glu Gly Ile Gly Tyr Asn Ile Ile Arg Val
Pro Met Ala Ser Cys Asn Phe Ser Ile Arg Thr Tyr Thr Tyr Ala Asp
Thr Pro Asn Asn Phe Gln Leu His Asn Phe Ser Leu Pro Glu Glu Asp
Thr Lys Leu Lys Ile Pro Leu Ile His Arg Ala Leu Gln Leu Ala Gln
Arg Pro Val Ser Leu Leu Ala Ser Pro Trp Thr Ser Pro Thr Trp Leu
Lys Thr Asn Gly Ala Val Asn Gly Lys Gly Ser Leu Lys Gly Gln Pro
Gly Asp Ile Tyr His Gln Thr Trp Ala Arg Tyr Phe Val Lys Phe Leu
Asp Ala Tyr Ala Glu His Lys Leu Gln Phe Trp Ala Val Thr Ala Glu
Asn Glu Pro Ser Ala Gly Leu Leu Ser Gly Tyr Pro Phe Gln Cys Leu
Gly Phe Thr Pro Glu His Gln Arg Asp Phe Ile Ala Arg Asp Leu Gly
Pro Thr Leu Ala Asn Ser Thr His His Asn Val Arg Leu Leu Met Leu
Asp Asp Gln Arg Leu Leu Leu Pro His Trp Ala Lys Val Val Leu Thr
Asp Pro Glu Ala Ala Lys Trp Val His Gly Ile Ala Val His Trp Tyr
Leu Asp Phe Leu Ala Pro Ala Lys Ala Thr Leu Gly Glu Thr His Arg
Leu Phe Pro Asn Thr Met Leu Phe Ala Ser Glu Ala Cys Val Gly Ser
Lys Phe Trp Glu Gln Ser Val Arg Leu Gln Ser Trp Asp Arg Gly Met
Gln Tyr Ser His Ser Ile Ile Thr Asn Leu Leu Tyr His Val Val Gly
Trp Thr Asp Trp Asn Leu Ala Leu Asn Pro Glu Gly Gly Pro Asn Trp
Val Arg Asn Phe Val Asn Ser Pro Ile Ile Val Asp Ile Thr Lys Asp
Thr Phe Tyr Lys Gln Pro Met Phe Tyr His Leu Gly His Phe Ser Lys
Phe Ile Pro Glu Gly Ser Gln Arg Val Gly Leu Val Ala Ser Gln Lys
Asn Asp Leu Asp Ala Val Ala Leu Met His Pro Asp Gly Ser Ala Val
Val Val Val Leu Asn Arg Ser Ser Lys Asp Val Pro Leu Thr Ile Lys
Asp Pro Ala Val Gly Phe Leu Glu Thr Ile Ser Pro Gly Tyr Ser Ile
His Thr Tyr Leu Trp Arg Arg Gln

- 17 -

1 6. A pharmaceutical composition comprising
2 therapeutic amount of the glycoprotein of claim 4, and
3 pharmaceutically acceptable carrier.

4 7. A method of treating Gaucher's disease,
5 comprising administering to a subject inflicted with
6 Gaucher's disease, therapeutic amount of the glycoprotein
7 of claim 4 to alleviate Gaucher's disease.

8 8. The clone of claim 1 having the
9 characteristics of ATCC 40393.

1/7

G	GAG	TTT	TCA	AGT	CCT	TCC	AGA	GAG	GAA	TGT	CCC	AAG	CCT	TTG	AGT	46
				Met	Ala	Gly	Ser	Leu	Thr	Gly	Leu	Leu	Leu	Leu	Gln	12
47	AGG	GTA	AGC	ATC	ATG	GCT	GGC	AGC	CTC	ACA	GGA	TTG	CTT	CTA	CTT	94
13	Ala	Val	Ser	Trp	Ala	Ser	Gly	Ala	Arg	Pro	Cys	Ile	Pro	Lys	Ser	28
95	GCA	GTG	TCG	TGG	GCA	TCA	GGT	GCC	CGC	CCC	TGC	ATC	CCT	AAA	AGC	142
29	Gly	Tyr	Ser	Ser	Val	Val	Cys	Val	Cys	Asn	Ala	Thr	Tyr	Cys	Asp	44
143	GGC	TAC	AGC	TCG	GTG	GTG	TGT	GTC	TGC	AAT	GCC	ACA	TAC	TGT	GAC	190
45	Phe	Asp	Pro	Pro	Thr	Phe	Pro	Ala	Leu	Gly	Thr	Phe	Ser	Arg	Tyr	60
191	TTT	GAC	CCC	CCG	ACC	TTT	CCT	GCC	CTT	GGT	ACC	TTC	AGC	CGC	TAT	238
61	Ser	Thr	Arg	Ser	Gly	Arg	Arg	Met	Glu	Leu	Ser	Met	Gly	Pro	Ile	76
239	AGT	ACA	CGC	AGT	GGG	CGA	CGG	ATG	GAG	CTG	AGT	ATG	GGG	CCC	ATC	286
77	Ala	Asn	His	Thr	Gly	Thr	Gly	Leu	Leu	Leu	Thr	Leu	Gln	Pro	Glu	92
287	GCT	AAT	CAC	ACG	GGC	ACA	GGC	CTG	CTA	CTG	ACC	CTG	CAG	CCA	GAA	334
93	Lys	Phe	Gln	Lys	Val	Lys	Gly	Phe	Gly	Gly	Ala	Met	Thr	Asp	Ala	108
335	AAG	TTC	CAG	AAA	GTG	AAG	GGA	TTT	GGA	GGG	GCC	ATG	ACA	GAT	GCT	382
109	Ala	Leu	Asn	Ile	Leu	Ala	Leu	Ser	Pro	Pro	Ala	Gln	Asn	Leu	Leu	124
383	GCT	CTC	AAC	ATC	CTT	GCC	CTG	TCA	CCC	CCT	GCC	CAA	AAT	TTG	CTA	430
125	Lys	Ser	Tyr	Phe	Ser	Glu	Glu	Gly	Ile	Gly	Tyr	Asn	Ile	Ile	Arg	140
431	AAA	TCG	TAC	TTC	TCT	TCT	GAA	GAA	GGA	ATC	GGA	TAT	AAC	ATC	CGG	478

FIG. 1-1

141	Pro	Met	Ala	Ser	Cys	Asp	Phe	Ser	Ile	Arg	Thr	Tyr	Thr	Tyr	Ala	Asp	156
479	CCC	ATG	GCC	AGC	TGT	GAC	TTC	TCC	ATC	CGC	ACC	TAC	ACC	TAT	GCA	GAC	526
157	Thr	Pro	Asp	Asp	Phe	Gln	Leu	His	Asn	Phe	Ser	Leu	Pro	Glu	Glu	Asp	172
527	ACC	CCT	GAT	GAT	TTC	CAG	TTG	CAC	AAC	TTC	AGC	CTC	CCA	GAG	GAA	GAT	574
173	Thr	Lys	Leu	Lys	Ile	Pro	Leu	Ile	His	Arg	Ala	Leu	Gln	Leu	Ala	Gln	188
575	ACC	AAG	CTC	AAG	ATA	CCC	CTG	ATT	CAC	CGA	GCC	CTG	CAG	TTG	GCC	CAG	622
189	Arg	Pro	Val	Ser	Leu	Leu	Ala	Ser	Pro	Trp	Thr	Ser	Pro	Thr	Trp	Leu	204
623	CGT	CCC	GTT	TCA	CTC	CTT	GCC	AGC	CCC	TGG	ACA	TCA	CCC	ACT	TGG	CTC	670
205	Lys	Thr	Asn	Gly	Ala	Val	Asn	Gly	Lys	Gly	Ser	Leu	Lys	Gly	Gln	Pro	220
671	AAG	ACC	AAT	GGA	GCG	GTG	AAT	GGG	AAG	GGG	TCA	CTC	AAG	GGA	CAG	CCC	718
221	Gly	Asp	Ile	Tyr	His	Gln	Thr	Trp	Ala	Arg	Tyr	Phe	Val	Lys	Phe	Leu	236
719	GGA	GAC	ATC	TAC	CAC	CAG	ACC	TGG	GCC	AGA	TAC	TTT	GTG	AAG	TTC	CTG	766
227	Asp	Ala	Tyr	Ala	Glu	His	Lys	Leu	Gln	Phe	Trp	Ala	Val	Thr	Ala	Glu	252
767	GAT	GCC	TAT	GCT	GAG	CAC	AAG	TTA	CAG	TTC	TGG	GCA	GTG	ACA	GCT	GAA	814
253	Asn	Glu	Pro	Ser	Ala	Gly	Leu	Leu	Ser	Gly	Tyr	Pro	Phe	Gln	Cys	Leu	268
815	AAT	GAG	CCT	TCT	GCT	GGG	CTG	TTG	AGT	GGA	TAC	CCC	TTC	CAG	TGC	CTG	862
269	Gly	Phe	Thr	Pro	Glu	His	Gln	Arg	Asp	Phe	Ile	Ala	Arg	Asp	Leu	Gly	284
863	GGC	TTC	ACC	CCT	GAA	CAT	CAG	CGA	GAC	TTC	ATT	GCC	CGT	GAC	CTA	GGT	910
285	Pro	Thr	Leu	Ala	Asn	Ser	Thr	His	His	Asn	Val	Arg	Leu	Leu	Met	Leu	300
911	CCT	ACC	CTC	GCC	AAC	AGT	ACT	CAC	CAC	AAT	GTC	CGC	CTA	CTC	ATG	CTG	958

FIG. 1-2

3/7

301	Asp	Asp	Gln	Arg	Leu	Leu	Leu	Pro	His	Trp	Ala	Lys	Val	Val	Leu	Thr	316
959	GAT	GAC	CAA	CGC	TTG	CTG	CTG	CCC	CAC	TGG	GCA	AAG	GTG	GTA	CTG	ACA	1006
317	Asp	Pro	Glu	Ala	Ala	Lys	Tyr	Val	His	Gly	Ile	Ala	Val	His	Trp	Tyr	332
1007	GAC	CCA	GAA	GCA	GCT	AAA	TAT	GTT	CAT	GGC	ATT	GCT	GTA	CAT	TGG	TAC	1054
333	Leu	Asp	Phe	Leu	Ala	Pro	Ala	Lys	Ala	Thr	Leu	Gly	Glu	Thr	His	Arg	348
1055	CTG	GAC	TTT	CTG	GCT	CCA	GCC	AAA	GCC	ACC	CTA	GGG	GAG	ACA	CAC	CGC	1102
349	Leu	Phe	Pro	Asn	Thr	Met	Leu	Phe	Ala	Ser	Glu	Ala	Cys	Val	Gly	Ser	364
1103	CTG	TTC	CCC	AAC	ACC	ATG	ATG	CTC	TTC	GCC	TCA	GAG	GCC	TGT	GTG	GGC	1150
365	Lys	Phe	Trp	Glu	Gln	Ser	Val	Arg	Leu	Gly	Ser	Trp	Asp	Arg	Gly	Met	380
1151	AAG	TTC	TGG	GAG	CAG	AGT	GTG	CGG	CTA	GGC	TCC	TGG	GAT	CGA	GGG	ATG	1198
381	Gln	Tyr	Ser	His	Ser	Ile	Ile	Thr	Asn	Leu	Leu	Tyr	His	Val	Val	Gly	396
1199	CAG	TAC	AGC	CAC	AGC	ATC	ATC	ACG	AAC	CTC	CTG	TAC	CAT	GTG	GTC	GGC	1246
397	Trp	Thr	Asp	Trp	Asn	Leu	Ala	Leu	Asn	Pro	Glu	Gly	Gly	Pro	Asn	Trp	412
1247	TGG	ACC	GAC	TGG	AAC	CTT	GCC	CTG	AAC	CCC	GAA	GGA	GGA	CCC	AAT	TGG	1294
413	Val	Arg	Asn	Phe	Val	Asp	Ser	Pro	Ile	Ile	Val	Asp	Ile	Thr	Lys	Asp	428
1295	GTG	CGT	AAC	TTT	GTC	GAC	AGT	CCC	ATC	ATT	GTA	GAC	ATC	ACC	AAG	GAC	1342

FIG. 1-3

477

429	Thr	Phe	Tyr	Lys	Gln	Pro	Met	Phe	Tyr	His	Leu	Gly	His	Phe	Ser	Lys	444
1343	ACG	TTT	TAC	AAA	CAG	CCC	ATG	TTC	TAC	CAC	CTT	GGC	CAC	TTC	AGC	AAG	1390
445	Phe	Ile	Pro	Glu	Gly	Ser	Gln	Arg	Val	Gly	Leu	Val	Ala	Ser	Gln	Lys	460
1391	TTC	ATT	CCT	GAG	GGC	TCC	CAG	AGA	GTG	GGG	CTG	GTT	GCC	AGT	CAG	AAG	1438
461	Asn	Asp	Leu	Asp	Ala	Val	Ala	Leu	Met	His	Pro	Asp	Gly	Ser	Ala	Val	476
1439	AAC	GAC	CTG	GAC	GCA	GTG	GCA	TTG	ATG	CAT	CCC	GAT	GGC	TCT	GCT	GTT	1486
477	Val	Val	Val	Leu	Asn	Arg	Ser	Ser	Lys	Asp	Val	Pro	Leu	Thr	Ile	Lys	492
1487	GTG	GTC	GTG	CTA	AAC	CGC	TCC	TCT	AAG	GAT	GTG	CCT	CTT	ACC	ATC	AAG	1534
493	Asp	Pro	Ala	Val	Gly	Phe	Leu	Glu	Thr	Ile	Ser	Pro	Gly	Tyr	Ser	Ile	508
1535	GAT	CCT	GCT	GTG	GGC	TTC	CTG	GAG	ACA	ATC	TCA	CCT	GGC	TAC	TCC	ATT	1582
509	His	Thr	Tyr	Leu	Trp	Arg	Arg	Gln									
1583	CAC	ACC	TAC	CTG	TGG	CGT	CGC	CAG	TGA	TGG	AGC	AGA	TAC	TCA	AGG	AGG	1630
1631	CAC	TGG	GCT	CAG	CCT	GGG	CAT	TAA	AGG	GAC	A						

FIG. 1-4

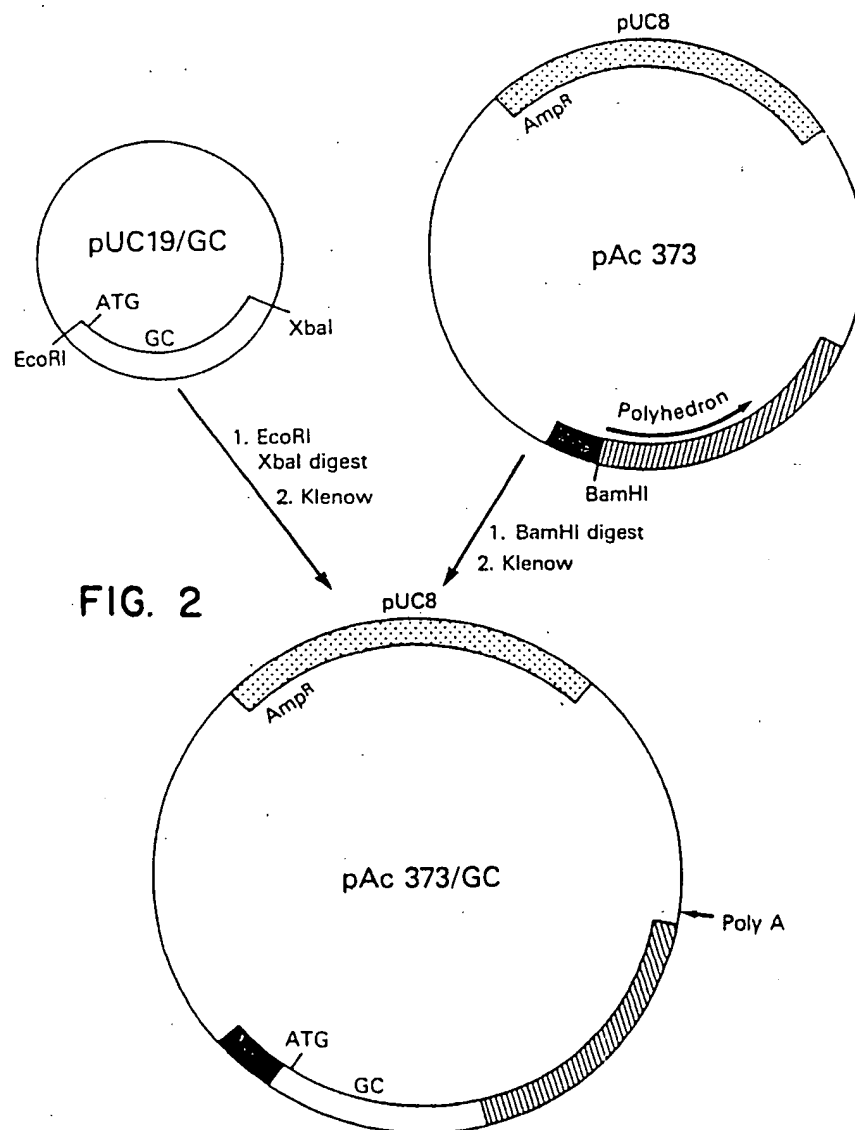


FIG. 2

6/7

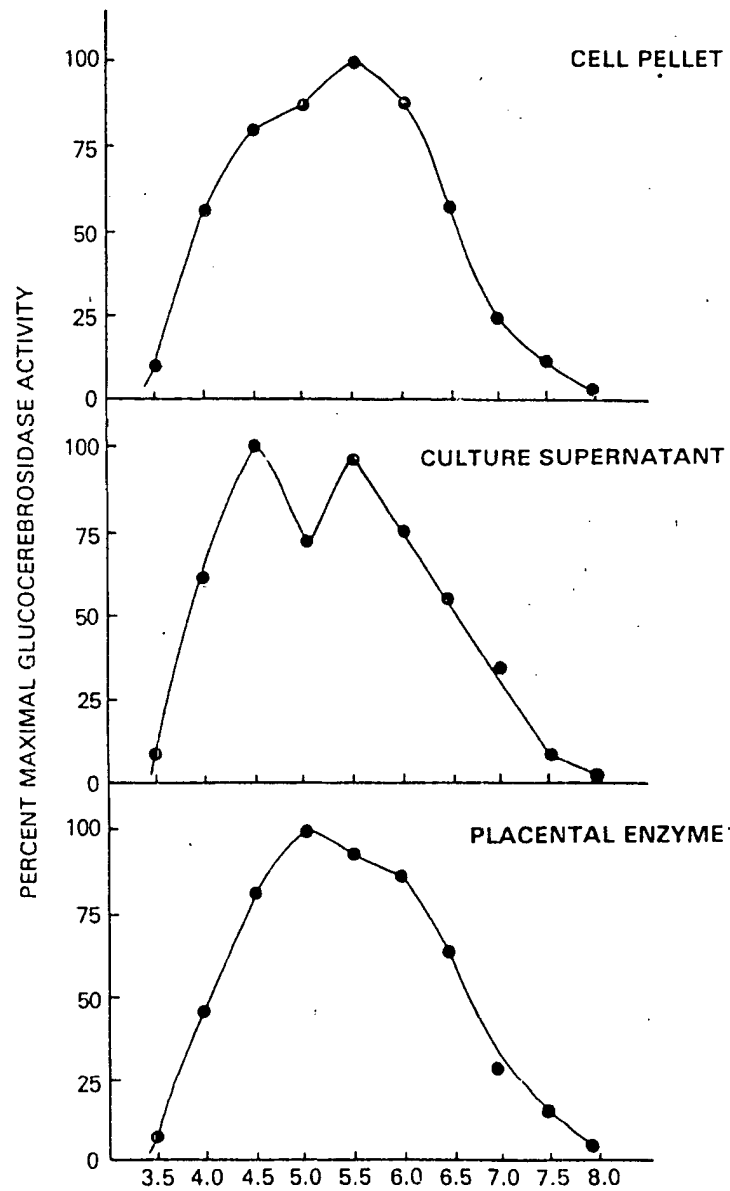


FIG. 3 pH

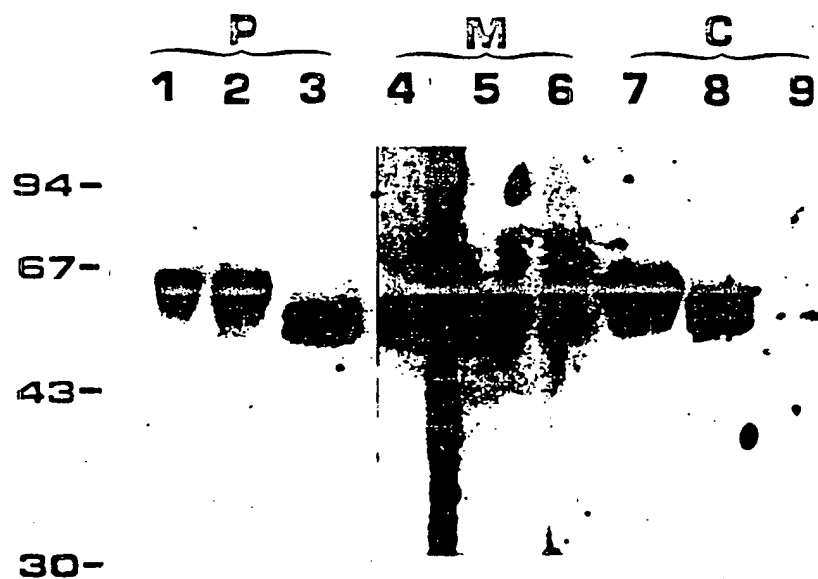


FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/04314

I. CLASSIFICATION OF SUBJECT MATTER (In several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to other National Classification: IPC
 IPC (4): C12N 7/00, 9/24, 5/00, 15/00; C07H 21/00; A61K 37/54
 U.S. Cl.: 536/27; 435/172.3, 200, 235, 240.2 424/94.61

II. FIELDS SEARCHED

Minimum Documentation Searched *

Classification System	Classification Symbols
U.S.	536/27; 435/68, 91, 172.3, 200, 235, 240.2, 320; 424/94.61; 935/32, 57, 70

Documentation Searched other than Minimum Documentation
 to the extent that such Documents are included in the Fields Searched *

Chemical Abstract Data Base (CAS) 1967-1989. Keywords:
 Baculovirus, Glucocerebrosidase, Vector, Gaucher, Recombin?

III. DOCUMENTS CONSIDERED TO BE RELEVANT *

Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	Proceedings of the National Academy of Sciences USA, Vol 82, issued November, 1985 (Washington, D.C.) by J. Sorge et al., "Molecular cloning and nucleotide sequence of human glucocerebrosidase cDNA", see pages 7289-7293. See particularly page 7291.	1, 5 and 8
Y	The Journal of Biological Chemistry, Volume 261, No. 1, issued January, 1986 (Baltimore, Maryland) by S. Tsuji et al, "Nucleotide Sequence of cDNA containing the complete coding sequence for human lysosomal Glucocerebrosidase", see pages 50-53. See particularly page 52.	1, 5 and 8
Y, P	US, A, 4,745,051 (SMITH) 17 May 1988. See entire document.	2 and 3

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"Z" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

Date of Mailing of this International Search Report

28 February 1989

29 MAR 1989

International Searching Authority

Signature of Authorized Officer

ISA/US

Mary E. Pratt
 Mary E. Pratt

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Journal of Biological Chemistry, Volume 260, No. 26, issued 15 November 1985 (Baltimore, Maryland) A. H. Erickson et al., "Biosynthesis of the Lysosomal Enzyme Glucocerebrosidase." See pages 14319-14324. See particularly 14321 and 14323.	4
Y		5, 6, and 7
Y	Journal of General Virology, Volume 68, Part 3, issued May 1987 (Great Britain) Y Matsuura et al., "Baculovirus Expression vectors: the requirements for High Level Expression of Proteins, Including Glycoproteins, "see entire document, pages 1233-1250.	2, 3, and 8
A	Journal of Virology, Volume 60, No. 3, issued March 1987 (Washington, D.C.), K.-T. Jeang et al., "Abundant Synthesis of Functional Human T-Cell Leukemia Virus Type I p40 ^x Protein in Eukaryotic Cells by using a Baculovirus Expression Vector", See pages 708-710.	2, 3, and 8
A	Proceedings of the National Academy of Sciences, USA, Volume 74, No. 8, issued August 1977 (Washington, D.C.) F.S. Furbish et al., "Enzyme replacement therapy in Gaucher's disease: Large-scale purification of glucocerebrosidase suitable for human administration", see p. 3560-3563.	6 and 7

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